VITROS Products Chemistry

INSTRUCTIONS FOR USE

CHOL

VITROS CHOL Slides

Cholesterol

Intended Use

For in vitro diagnostic use only.

VITROS CHOL Slides quantitatively measure cholesterol (CHOL) concentration in serum and plasma.

Summary and Explanation of the Test

Cholesterol is present in tissues and in plasma lipoproteins either as cholesterol or as cholesterol esters bound to proteins. Cholesterol is an essential structural component of cell membranes and the outer layer of plasma lipoproteins and is the precursor of all steroid hormones, including sex and adrenal hormones, bile acids, and vitamin D.

Cholesterol measurements are used to evaluate the risk of developing coronary artery occlusion, atherosclerosis, myocardial infarction, and cerebrovascular disease. Coronary atherosclerosis correlates with a high cholesterol level. Cholesterol concentrations are increased in primary hypercholesterolemia; secondary hyperlipoproteinemia, including nephrotic syndrome; primary biliary cirrhosis; hypothyroidism; and in some cases diabetes mellitus. Low cholesterol concentrations may be found in malnutrition, malabsorption, advanced malignancy, and hyperthyroidism. Serum cholesterol concentration depends on many factors, including age and gender. [1]

Principles of the Procedure

The VITROS CHOL Slide is a dry, multilayered, analytical element coated on a polyester support. The method is based on an enzymatic method similar to that proposed by Allain et al. [2]

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The Triton X-100 (TX100) surfactant in the spreading layer aids in dissociating the cholesterol and cholesterol esters from lipoprotein complexes present in the sample. Hydrolysis of the cholesterol esters to cholesterol is catalyzed by cholesterol ester hydrolase. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye.

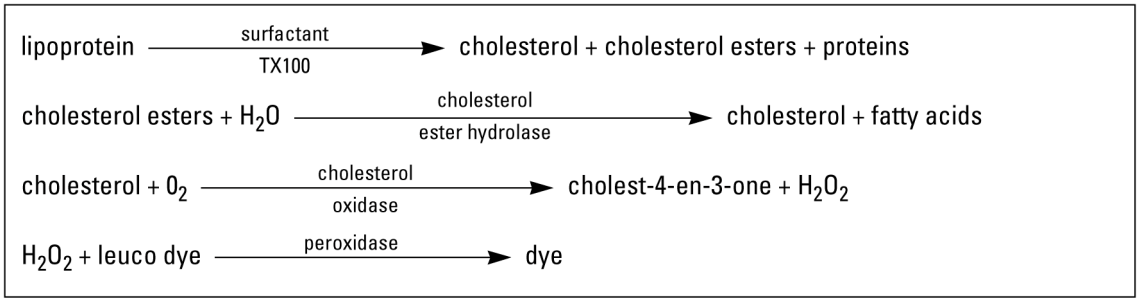
The density of dye formed is proportional to the cholesterol concentration present in the sample and is measured by reflectance spectrophotometry.

Test Type	Wavelength	Assay Time and Temperature
Colorimetric	540 ηm	Approximately 5 minutes at 37°C

Sample Drop Volume

The volume of the sample drop depends on the format of the slide. For slides with coatings labeled 3201 and above, the sample drop volume is 5.5 μL. For all other slide formats, the sample drop volume is 10 μL.

Reaction Sequence



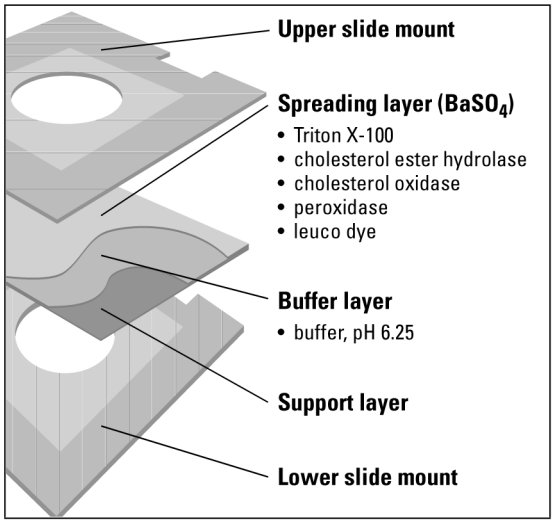
Reagents

Slide Ingredients

Reactive ingredients are Triton X-100; cholesterol oxidase (Nocardia or Cellulomonas, E.C.1.1.3.6); cholesterol ester hydrolase (Candida rugosa or Pseudomonas, E.C.3.1.1.13); peroxidase (horseradish root, E.C.1.11.1.7); and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis (4-dimethylaminophenyl) imidazole (leuco dye).

Other ingredients include pigment, binder, buffer, surfactants, stabilizers, and cross-linking agent.

Slide Diagram



Slide Labeling

The cartridge's outer carton is labeled with the test name, slide lot number, expiration date, and required storage temperature.

Slide Cartridge Handling

CAUTION:	**Protect the inner wrapper from damage before opening.**

- Do not drop a case of cartridges.
- Do not cut into the inner wrapper with a sharp instrument when opening the case.

Slide Storage

Unopened slide cartridges:

Gen 22 and below: Store at or below 2°–8°C (36°–46°F).

Gen 23 and above: Store at or below -18°C (0°F).

NOTE:	To reduce cartridge warm-up time or if freezer space is limited, unopened slide cartridges may be stored in the refrigerator at 2°–8°C (36–46°F) for up to six months.

Cartridges in the system's slide supply:

- Leave in the slide supply for no more than two weeks, then replace with a fresh cartridge.
- Leave in the slide supply when the system is turned off for up to two hours.
- Verify performance with control materials:
  - If the system is turned off for more than two hours
  - After reloading cartridges that have been removed from the slide supply and stored for later use

Slide Stability

VITROS CHOL Slides are stable until the expiration date on the carton when they are stored and handled as specified.

Slide Preparation

- Remove slide cartridges from storage.
- The slide cartridge must reach room temperature, 18°–28°C (64°–82°F), before it is unwrapped and loaded into the slide supply. Allow the cartridge to warm up at least:
  - 60 minutes after removing from the freezer

    or
  - 30 minutes after removing from the refrigerator
- Remove the inner wrapper and immediately load into the slide supply.

NOTE:	Load the cartridges within 24 hours after they reach room temperature.

Specimen Collection and Preparation

Patient Preparation

No special patient preparation is necessary.

Recommended Specimen Types

Serum; heparin plasma.

Specimens Not Recommended

Sodium citrate, EDTA, and sodium fluoride/potassium oxalate as anticoagulants.[3]

Specimen Collection and Preparation

- Collect specimens using standard laboratory procedures.[4, 5]
- Refer to the operator's manual section on sample handling for recommended minimum specimen volumes for your system.
- Centrifuge specimens and remove the serum from the clot within three hours of collection.[6]

Handling and Storage Conditions

- Handle specimens as biohazardous material.
- Handle specimens in stoppered containers to avoid contamination and evaporation.
- Storage requirements:[7]
  - Store refrigerated up to three days at 2°–8°C (36°–46°F).
  - Store frozen for up to three weeks at or below -18°C (0°F).
  - Storage at room temperature is not recommended.

Testing Procedure

Materials Required But Not Provided

The following items are required to perform the test for CHOL:

- VITROS Chemistry Calibrator Kit 2
- Quality-control materials, such as VITROS Performance Verifiers
- For dilution, VITROS 7% BSA

Operating Instructions

Refer to the operator's manual for complete instructions on operation of your system.

Sample Dilution

If samples are lipemic or show cholesterol concentrations that exceed the system's reportable (dynamic) range, follow this procedure.

1. Dilute 1 part sample with 1 part VITROS 7% BSA.
2. Reanalyze.
3. Multiply the results by 2 to obtain the original sample's cholesterol concentration.

Calibration

Required Calibrators

VITROS Chemistry Calibrator Kit 2

Calibrator Preparation, Handling, and Storage

Refer to the calibrator package insert for information about reconstitution and use of the Chemistry Calibrator Kit.

Calibration Procedure

Refer to the calibration section of your operator's manual.

When to Calibrate

- Calibrate when the slide lot number changes.
- Calibrate when critical system parts are replaced due to service or maintenance.
- If quality-control results are consistently outside acceptable limits, calibration might be required. Refer to your operator's manual for more detail.
- Calibrate when government regulations require. In the US, CLIA regulations require calibration or calibration verification at least once every six months.

Reference Method

Calibration is traceable to the method of Abell et al. [8]

Calibration Model

End-point colorimetry (described in your operator's manual).

Quality Control

Procedure Recommendations

- Handle quality-control materials as biohazardous material.
- Analyze quality-control materials in the same manner as patient samples, before or during patient sample processing.
- Analyze control materials at least once per day to verify system performance.
- Choose control levels that check the clinically relevant range.
- Refer to the quality control section in your operator's manual for additional information on quality-control procedures for VITROS Systems.
- Refer to *Internal Quality Control Testing: Principles and Definitions* for general quality-control recommendations.[9]

Quality-Control Material Selection

- VITROS Performance Verifiers are specially formulated for use with VITROS Systems.
- Other control materials may show a difference when compared with other cholesterol methods if they:
  - Depart from a true human serum/plasma matrix
  - Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives
- Do not use control materials stabilized with ethylene glycol.

Quality-Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Expected Values and Reporting Results

Reference Values [10]

Concentration	Conv. Units (mg/dL)	SI Units (mmol/L)	Alternate Units (g/L)
Desirable	< 200	< 5.2	< 2.0
Borderline High	200–239	5.2–6.2	2.0–2.4
High	> 240	> 6.2	> 2.4

Reporting Units and Unit Conversion

Conventional Units	SI Units	Alternate Units
mg/dL	mmol/L (mg/dL x 0.02586)	g/L (mg/dL x 0.01)

Limitations of the Procedure

Known Interfering Substances

The VITROS CHOL method was screened for interfering substances following NCCLS Protocol EP7. [11] The following substances, when tested at the concentrations indicated, caused the bias shown.

	Conventional Units				SI Units		
Interferent*	Interferent Conc. (mg/dL)	Comments	Analyte Conc. (mg/dL)	Average Bias** (mg/dL)	Interferent Conc. (mmol/L)	Analyte Conc. (mmol/L)	Average Bias** (mmol/L)
Gentisic acid	5.0	Upper Therapeutic	230	-31	0.32	5.9	-0.80
N-acetylcysteine	10.0	Therapeutic, Oral	230	-26	0.61	5.9	-0.67

* It is possible that other interfering substances may be encountered. These results are representative; however, your results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed might not be predictable.

** The bias is an estimate of the maximum difference observed.

Other Limitations

Some drugs and patient conditions are known to alter cholesterol concentration in vivo. A compilation of this information is available in the literature. [12, 13]

Performance Characteristics

Reportable Range (Dynamic Range)

Conv. Units (mg/dL)	SI Units (mmol/L)	Alternate Units (g/L)
50–325	1.29–8.40	0.50–3.25

Refer to Sample Dilution under “Testing Procedure” for out-of-range samples.

Sensitivity

The lower limit of the reportable (dynamic) range is 50 mg/dL (1.29 mmol/L; 0.50 g/L).

Precision

Precision was evaluated with quality-control materials on VITROS 250, 700, and 950 Chemistry Systems following NCCLS Protocol EP5. [14]

These results are guidelines. Variables such as instrument maintenance, environment, slide handling/storage, control material reconstitution, and sample handling can affect the reproducibility of test results.

CHOL Precision

SYSTEM	Conventional Units (mg/dL)			SI Units (mmol/L)			Within Lab CV%**	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**			
VITROS 250	148	1.1	1.9	3.8	0.03	0.05	1.3	84	21
	248	2.3	2.7	6.4	0.06	0.07	1.1	84	21
VITROS 700	148	1.6	2.7	3.8	0.04	0.07	1.8	84	21
	249	3.3	5.1	6.4	0.09	0.13	2.0	84	21
VITROS 950	149	2.8	3.9	3.9	0.07	0.10	2.6	88	22
	249	4.8	6.3	6.4	0.13	0.16	2.5	88	22

* Within Day precision was determined using two runs/day with two to three replications.

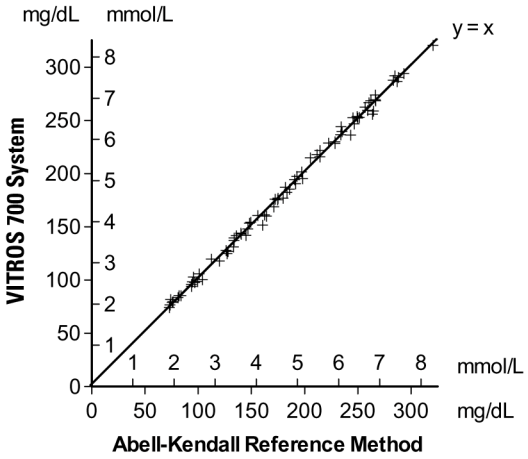
** Within Lab precision was determined using a single lot of slides and calibrating weekly.

Accuracy

The plot and table show the results of a comparison of serum specimens analyzed on the VITROS 700 System with those analyzed using the Abell-Kendall reference method[8] following NCCLS Protocol EP9. [15]

The table also shows the results of comparisons of the VITROS 250 and 950 Systems with the VITROS 700 System.

CHOL/Serum



Method Comparison (Serum)

				Conventional Units (mg/dL)			SI Units (mmol/L)		
	n	Slope	Correlation Coefficient	Range of Sample Concentration	Intercept	Sy.x	Range of Sample Concentration	Intercept	Sy.x
700 System vs. reference method8	79	1.00	0.998	73–321	0.14	3.91	1.89–8.30	0.004	0.10
250 System vs. 700 System	73	1.00	1.000	75–309	0.10	1.90	1.94–7.99	0.003	0.05
950 System vs. 700 System	73	1.00	1.000	75–309	0.06	1.38	1.94–7.99	0.002	0.04
250 System vs. 950 System	73	1.00	1.000	75–309	0.12	2.28	1.94–7.99	0.003	0.06
250 System vs. Commercially available enzymatic assay	497	0.97	0.994	66–319	4.20	4.92	1.71–8.25	0.109	0.13

Specificity

The following substances were tested with VITROS CHOL Slides following NCCLS Protocol EP7[16] and found not to interfere (bias ≤ 10.4 mg/dL or ≤ 0.27 mmol/L) at a CHOL concentration of 230 mg/dL (5.95 mmol/L):

Compound	Concentration	
Acetaminophen	400 μg/mL	2646 μmol/L
Alprazolam	200 ηg/mL	647.7 ηmol/L
Amitriptyline	1 μg/mL	3.6 μmol/L
Amoxicillin	1500 μg/mL	4.1 mmol/L
Ascorbic Acid	3 mg/dL	170.4 μmol/L
Atenolol	2 μg/mL	7.5 μmol/L
2-Benzothiazolethiol	5 mg/dL	0.3 mmol/L
Bilirubin	20 mg/dL	341.9 μmol/L
Carbamazepine	60 μg/mL	254.0 μmol/L
Cephalexin	400 μg/mL	1.2 mmol/L
Chlorothiazide	10 mg/dL	338.2 μmol/L
Ciprofloxacin	5 mg/dL	150.9 μmol/L
Codeine	4 μg/mL	13.4 μmol/L
D-thyroxine	375 μg/dL	4838 ηmol/L
7-Dehydrocholesterol	20 mg/dL	0.52 mmol/L
Dextran 40	1000 mg/dL	25.0 μmol/L
Dextromethorphan	3.8 μg/mL	14.0 μmol/L
Diflunisal	50 mg/dL	2.0 mmol/L
Dihydrocholesterol	5 mg/dL	0.13 mmol/L
Diltiazem	5 μg/mL	12.1 μmol/L
Diphenhydramine	10 μg/mL	39.1 μmol/L
Dipyrone	6 mg/dL	205.2 μmol/L
Enalapril	1.2 μg/mL	3.2 μmol/L
Estradiol	27 μg/mL	100.2 μmol/L
Ethamsylate	3 mg/dL	113.9 μmol/L
Ethanol	394 mg/dL	85.6 mmol/L
Furosemide	10 mg/dL	302.3 μmol/L
Glucose	1200 mg/dL	66.6 mmol/L
Glutathione	1 mg/dL	32.5 μmol/L
Glybenclamide	6.4 μg/mL	13.0 μmol/L
Guaifenesin	100 mg/dL	5.0 mmol/L
Hemoglobin	150 mg/dL	93.0 μmol/L

Compound	Concentration	
Hydrochlorothiazide	2 mg/dL	67.2 μmol/L
Hypaque	500 mg/dL	8.2 mmol/L
Ibuprofen	40 mg/dL	1.9 mmol/L
Isoniazid	7 mg/dL	510.6 μmol/L
L-Dopa	0.6 mg/dL	30.4 μmol/L
L-thyroxine	375 μg/dL	4838 ηmol/L
6-Mercaptopurine	10 mg/dL	656.9 μmol/L
Methyldopa	0.5 mg/dL	23.7 μmol/L
Metoprolol	0.34 mg/dL	6.4 μmol/L
Naproxen	900 μg/mL	3.9 mmol/L
Nicotinic Acid	100 mg/dL	8.1 mmol/L
Nifedipine	2 μg/mL	5.8 μmol/L
p-Aminosalicylic Acid	25 mg/dL	1.9 mmol/L
Phenol	10 mg/dL	1.0 mmol/L
Phenytoin	10 mg/dL	396.4 μmol/L
Phospholipids	400 mg/dL	400 g/L
Prednisone	1 μg/mL	2.8 μmol/L
Propoxyphene	0.4 mg/dL	11.8 μmol/L
Ranitidine	20 μg/mL	63.8 μmol/L
Salicylate	50 mg/dL	3.6 mmol/L
Streptomycin	30 mg/dL	263.5 μmol/L
Sulfamethoxazole	33 mg/dL	130.3 μmol/L
Sulfathiazole	35 mg/dL	1.4 mmol/L
Sulfinpyrazone	40 mg/dL	98.9 μmol/L
Terazosin	1 mg/dL	25.8 μmol/L
Total Protein	9 g/dL	90 g/L
Triglycerides	600 mg/dL	6.8 mmol/L
Trimethoprim	25 mg/dL	861.1 mmol/L
Tyrosine	24 mg/dL	1325 mmol/L
Urea	462 mg/dL	76.9 mmol/L
Verapamil	90 μg/mL	197.7 μmol/L
Warfarin	100 μg/mL	323.9 μmol/L

References

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Revision History

Date of Revision:	Version:	Description:
2002APR19	1.0	New format. Update to 2000MAR06: Principles of the Procedure—Added a Sample Drop Volume subsection Sensitivity References

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

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Signature Obsolete Date

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